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A STUDY ON FOUR ANTIOXIDATION EFFECTS OF *LYCIUM BARBARUM* POLYSACCHARIDES *IN VITRO*

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Abstract

The objective of the study was to investigate the in vitro antioxidation activity of *lycium barbarum* polysaccharides (LBP). Ultraviolet spectrophotometry was adopted to determine the capability of LBP to clear superoxide anions, hydroxyl radicals, DPPH free radicals and ABTS free radicals. The result showed that the law for LBP to clear superoxide anions, hydroxyl radicals and DPPH free radicals was that the clearance rate increased gradually with the increase of the concentration, and when the concentration reached a certain value, the clearance rate leveled off, while the IC_{50} for clearing ABTS free radicals was 47.158±6.231μg/ml. The study concluded that LBP is a good *in vitro* antioxidant.

Keywords: LBP; superoxide anion; hydroxyl radical; DPPH; ABTS

Introduction

Barbary wolfberry fruit is the dry and ripe fruit of *Lycium barbarum* in Solanaceae plant, with the function of nourishing liver and kidney, and it is one of medicinal and edible substances. Its main chemical constituents are LBP, betaine, vitamins, trace elements, etc. LBP is one of the main active ingredients and has anti-tumor, anti-aging, antioxidation, immune function regulation, blood sugar lowering, blood fat lowering (Li et al., 2007; Chen et al., 2008; Luo et al., 2004) and other functions. In this paper, four different methods are adopted to study the capability of LBP to clear free radicals, so as to reflect its antioxidation effect and lay a foundation for the study on the functional foods made of barbary wolfberry.

Materials and Methods

Drug, reagents and Instruments

Drugs and reagents included the following: LBP (Shanghai Kangzhou Fungi Extract Co., Ltd), phenanthroline (Tianjin Beifang Tianyi Chemical Reagent Factory), hydrogen peroxide (Shandong Dezhou Anjie High-Tech Disinfection Products Co., Ltd), ferrous sulfate (Tianjin Hengxing Chemical Reagent Co., Ltd), absolute ethanol and methanol (Analytical pure, Tianjin Kermel Chemical Reagent Co., Ltd), and potassium persulfate (Tianjin Jinhui Taiya Chemical Reagent Co., Ltd). HH-8 digital thermostatic water bath (Guohua Electric Appliance Co., LTD) was used.

Methods were carried out through hydroxyl radical generation and its clearance rate determination as described by Jin, 2006. We weighed 1 g of phenanthroline and prepared it into 75mmol/L absolute ethanol solution. It was then diluted 10 times. $FeSO_4$, H_2O_2, and different concentrations of sample solution were prepared for use.

·OH generation model establishment

Precisely, 2.0ml 150mmol/L pH7.4 PBS, 0.2ml 7.5mmol/L phenanthroline, 0.2ml 7.5mmol/L $FeSO_4$, 0.4ml drug of different concentrations, 0.8ml distilled water and 0.4ml 1% H_2O_2, were measured. They were added into a test tube and mixed as the sample group. For the test group, solvent replaced the drug, and the other steps were same as the sample group. For the blank control group, solvent and distilled water replaced the drug and 1% H_2O_2 respectively. The other steps were same as the sample group. The test tubes were simultaneously placed

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into 37 °C water bath for 60min and their absorbance values were measured at 536 nm. •OH clearance rate was calculated using to the following formula:

$S_{\cdot OH}(\%)=(A_{sample}-A_{damage})/(A_{blank}-A_{damage}) \times 100\%$.

to draw the logarithm of clearance rate (Y) to drug concentration (X) for the linear equation, and get SC50 according to the linear equation.

Superoxide anion generation and its clearance rate determination (Jin, 2006)

4.5ml 0.05mmol/L pH8.2 Tris-Hcl buffer was added into a test tube and warmed up at 25 ℃ for 20min. 0.2ml 0.1mmol/L pyrogallol, 0.2ml drug of different concentrations and 0.1ml distilled water, were added. Water bath reaction at 25℃ for 4min was observed. The absorbance value was measured at 325nm and denoted as A_{sample}. Distilled water replaced the drug, and the other steps were same as above, and denoted as A_{damage}. Distilled water replaced the drug and Tris-Hcl buffer, and the other steps were same as above, denoted as A_{blank}.

The clearance rate was determined according to the following formula:

$S_{O2\cdot-}(\%) = (A_{damage}-A_{sample})/(A_{damage}-A_{blank}) \times 100\%$.

The linear equation and SC_{50} according to the linear equation were calculated to draw the logarithm of clearance rate (Y) to drug concentration (X).

DPPH free radical clearance test

We referred to the methods in the literature (Zheng et al., 2010; Li et al., 2006b) and made minor modifications. Precisely, DPPH powder was weighed, dissolved with methanol, and prepared into 6mmol/L solution for later use. It was diluted with methanol to 60μmol/L before use. In the reaction system, 50μL sample (methanol solution) of different concentrations and 3mL 60μmol/L DPPH methanol solution were placed in dark in 30℃ water bath for 30min after shaking, and then the absorbance value was measured at 515nm. The inhibition rate was calculated using the following formula: DPPH free radical clearance rate (I%) = $(1-A_S/A_0) \times 100\%$, in which, A_S is the absorbance value of sample; A_0 is the absorbance value of the reference product.

ABTS free radical clearance test

We referred to the methods in the literature (Foroogh et al, 2008; Chun et al, 2005; Lee et al, 2006) and made minor modifications. For the preparation of the ABTS solution: potassium persulfate was prepared into 2.45mmol/l water solution, dissolved ABTS with potassium persulfate, prepared into 7mmol/l ABTS stock solution, and placed in dark at room temperature from 12~16h for later use. It was then diluted with absolute ethanol before use, and in fact, the absorbance at 734nm was 0.70±0.02. Determination: 2.9ml diluted ABTS solution and 0.1ml sample or ethanol solution of reference product were taken and mixed. It was placedat 30℃ in the dark for 10min, and then the absorbance was measured at 734nm:

ABTS free radical clearance rate (%) = $(1-A_S/A_0) \times 100\%$.

Results

Result of hydroxyl radical clearance rate

This experiment respectively tested the inhibition effect of different concentrations of LBP on hydroxyl radicals. Three parallels were made for each concentration gradient. The mean values were taken to calculate the clearance rate, and the results are shown in the figure 1. The figure shows that LBP concentration is in the range of 10~250μg/ml. Its concentration and clearance rate show good dose-effect relationship. The clearance rate increases gradually with the increase of concentration, and the highest clearance rate is 89.45%.

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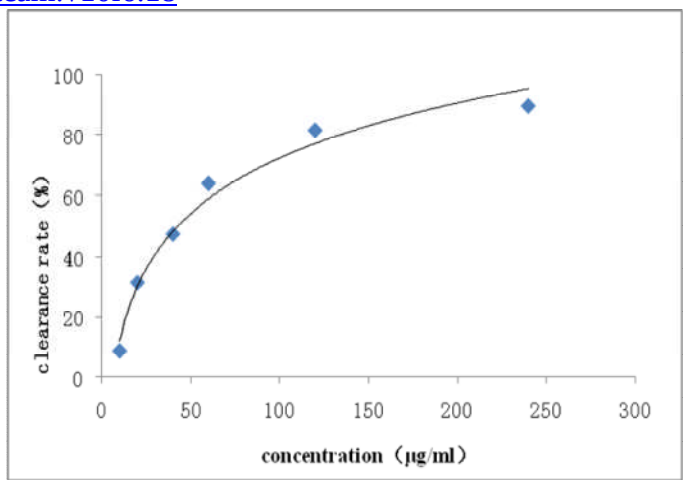

Figure 1: Dose-effect relationship of LBP clearing hydroxyl radicals

Within the concentration range of 10~60μg/ml, the clearance rate increases rapidly. It is calculated that IC_{50} of LBP to hydroxyl radical is 6.45μg/ml.

Result of superoxide anion clearance rate

The superoxide anion clearance rate of LBP of difference concentrations is measured as shown in the figure 2.

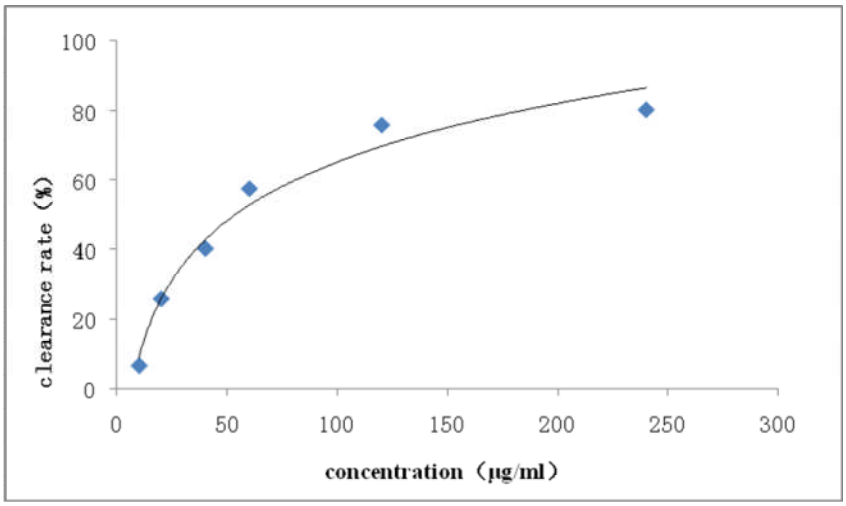

Figure 2: The superoxide anion clearance rate of LBP of difference concentrations

The figure shows that within the concentration range of 10~250μg/ml, LBP has good inhibition effect. It is calculated that IC_{50} is 7.13μg/ml.

Result of DPPH free radical clearance rate

Free radical clearing ability is expressed with clearance rate, and the higher the clearance rate, the stronger the antioxidation capacity. The result of clearing ability of different concentrations of LBP, VC and BHT to DPPH free radicals is shown in Figure 3. The result shows that as LBP concentration increases, the clearance rate increases gradually, and when the concentration increases to a certain value, the clearance rate levels off, same with the trend of positive drugs.

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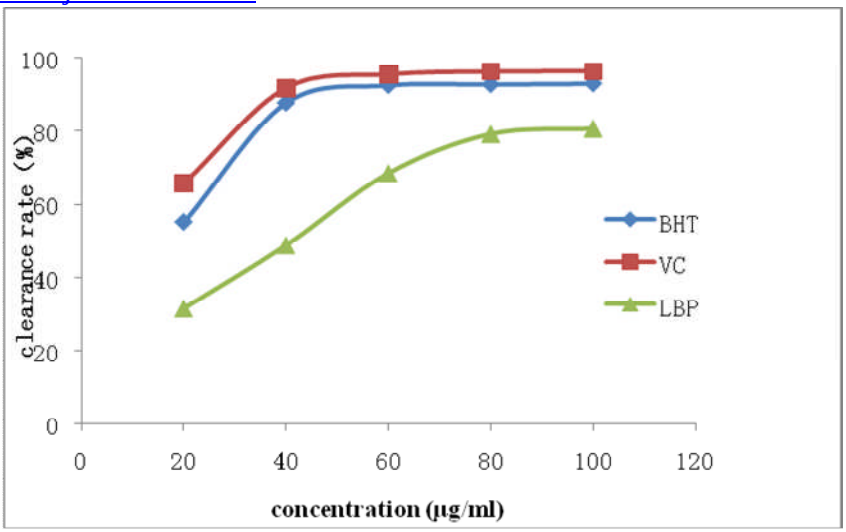

Figure 3: The result of DPPH free radical clearance rate

Result of ABTS free radical clearance rate

Table 1: Result of ABTS free radical clearance rate

Name	IC_{50}(μg/ml)
LBP	47.158±6.231
BHA	32.627±1.043
Trolox	2.356±0.023

Note: the data in the table are mean ± standard deviation (n=3)

The result shows that IC_{50} of LBP clearing ABTS free radicals is 47.158±6.231μg/ml.

Discussion

The Harman proposed the free radical theory of aging that in the long-term evolution of organism, free radicals in body are at an equilibrium state, free radicals have a high degree of chemical reactivity, free radicals cause lipid peroxidation which can denature nucleic acids, proteins and other macromolecular components, resulting in the alteration and destruction of cell structure, and if they cannot be effectively cleared, they will cause aging, cancer, cardiovascular disease and other degenerative diseases. However, endogenous antioxidant defense mechanism cannot be completely effective, so dietary antioxidants are more important to oxidative damage in the body (Li et al., 2006a). Lycium, as a medicinal and edible material, can effectively clear superoxide anions, hydroxyl radicals and DPPH and ABTS free radicals, consistent with the report in literature (Wang et al., 2011).

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References

1. Chen ZS, Kwong B, Tan H, Chan SH. (2008) Activation of T lymphocytes by polysaccharide-protein complex from Lycium barbarum L. International Immunopharmacology, **8(12)**: 1663-1671
2. Chun SS, Vattem DA, Lin YT, Kalidas S. (2005) Phenolic antioxidants from clonal oregano (Origanum vulgare) with antimicrobial activity against Helicobacter pylori. Process Biochemistry, **40**: 809-816
3. Foroogh B, Abbas K. (2008) Antioxidant activity and phenolic content of various date palm (Phoenix dactylifera) fruits from Iran. Food chemistry, **107**: 1636-1641

http://dx.doi.org/10.4314/ajtcam.v10i6.18
4. Jin Y. (2006) Study on the structure activity relationship of quercetin and its uniglucoside isoquercetin and diglucoside rutin resisting free radicals. Dalian Medical University.
5. Lee IK, Yun BS. (2006) Hispidin analogs fron the mushroom Inonotus xeranticus and their free radical scavenging activity. Bioorganic&Medicinal Chemistry Letters, **16**: 2376-2379
6. Li CX, Jiao Y, Liang QQ, Feng L, An HG. (2006a) Study on antioxidation activity of lycium ruthenicum extract. Science and Technology of Food Industry, **27(10)**: 55-57
7. Li XL, Zhou AG. (2006b) Evaluation of the antioxidant effecr of polysaccharides extracted of from Lycium barbarum. Medicinal Chemistry Research, **15(9)**: 471-482
8. Li XM, Ma YL, Liu XJ. (2007) Effect of the Lycium barbarum polysaccharides on age-related oxidative stress in aged mice. Journal of Ethnopharmacology, **111(3)**: 504-511
9. Luo Q, Cai YZ, Yan J, Sun M, Corke H. (2004) Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. Life Sciences, **76(2)**: 137-149
10. Wang XG, Cao YL. (2011) Lycium antioxidation function study and progress. Ningxia Journal of Agriculture and Forestry Science and Technology, **52(11)**: 48-52
11. Zheng SY, Chen TF, Zheng WJ. (2010) Spectroscopic study on single-cluster tea water extract clearing DPPH and ABTS free radicals. Spectroscopy and Spectral Analysis, **30(9)**: 2417-2423